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FEATURES OF MICROELEMENT BALANCE IN THE BRAIN TISSUES OF RATS UNDER EXPERIMENTAL HYPOXIA OF VARIOUS SEVERITY

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Abstract

Features of essential microelements (iron, zinc, copper, manganese, and cobalt), conditionally toxic chromium and toxic lead content were studied in tissues of cerebrum of newborn rats under the experimental hypoxia of various severity. Tissues of newborn rats cerebrum are characterized by a high level of saturation and considerable dynamism in the content of microelements (iron, zinc, copper, chromium, manganese). Until the end of the first week of life, the content of these microelements decreases 1.5-10 times. The level of toxic lead goes down more than 2.5 times. On the contrary, cobalt is an exception; its content increases almost 1.2 times.

The mild hypoxia leads to a 3 times decrease of cobalt level in newborn rats, causes almost 2 times decline of the iron level, and diminishes the levels of manganese by 27.65%, chromium – by 25.84%, zinc – by 16.43%, thus signifying to the onset of a considerable deficiency and imbalance of the microelements content in tissues of cerebrum. The severe hypoxia is characterized by further increase of microelements deficiency and imbalance.

Keywords: *hypoxia, cerebrum, microelements, newborn rats.*

Introduction

Perinatal hypoxia is the basic issue of neonatology. This is caused by its rank in disease structure, perinatal mortality, and multisystem disorder formation effects [*Lishya L. et al., 2008; Feala J. et al., 2009; Kadam S. et al., 2010*]. The most common consequence of perinatal hypoxia is central nervous system (CNS) damage, which according to many scientists is diagnosed in 60-80% of cases [*Xu F. et al., 2012*]. The CNS damages can occur because of some complicated neurochemical processes, such as lactate accumulation, phospholipase activization, excited amino acid emission, arachidonic acid stage energization, vasoactive substance educing, oxygen chemical groups and hydroperoxides energization [*Donna M., Feriero M., 2004; Vasiljevic B. et al., 2011*]. However, microelements assist the passage of many important biological reactions and act as contact substances (catalysts) for many of them.

As an essential microelement and structural component of proteins the iron takes part in the activity of enzymatic systems providing both systemic and cellular aerobic metabolism, as well as oxidation-reduction homeostasis of the organism [*Umamaheswari K., 2011*]. Regarding the iron content, cells of the brain are on the second place after hemoglobin of red blood cells. Metabolism of the iron in tissues of the brain is at a lower level than in the liver. The ability of depositing this microelement in the brain is decreased. In case of lowering iron levels the number and sensitivity of receptors is decreased; as a result, the metabolism of dopamine in nerve synapses and the number of pulses that pass though them is reduced [*Beard J., 2007*].

Manganese has vital functions for the brain activity: it ensures the normal structure and stability of the membranes and affects the metabolism of catecholamines [*Valko M. et al., 2005*]. The neurotropic effect of manganese is manifested in excita-

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tion of neurotransmitter systems. This microelement enhances the ion permeability of protoplasmatic membranes, leads to hyperpolarization of afferent fibers [*Wieringa F. et al., 2007*].

Numerous studies proved the vital biological role of zinc. It is indispensable in the biosynthesis of proteins (including collagen and poliribosomes) and during cell proliferation. Zinc has a stabilizing effect on epinephrine and norepinephrine, plays an important role in synaptic transmission in the CNS [*Wieringa F. et al., 2007*].

The biological role of copper is associated with its participation in the functioning of many enzymes, hormones, and vitamins. Copper significantly affects the growth and development of the body, different types of metabolism, hematopoiesis, skeleton formation. This element is the important functional component of various proteins (ceruloplasmin, cytochrome oxidase, superoxide dismutase, etc.) [*Prabodh S. et al., 2011*].

Cobalt is a part of vitamin B12 (cobalamin) molecule. The deficiency of cobalt is mostly noticeable in areas of rapid cell division, for example, in blood-forming tissues of the bone marrow and nerve structures. In addition, the body needs cobalt for stimulation of erythropoiesis. Cobalt reduces the activity of NADPH oxidase and thereby suppresses proteinuria [*Sabolic I., 2006; Ohtomo S. et al., 2008*].

The hexavalent chromium has neurotoxic, carcinogenic and allergic effects, but trivalent chromium has positive properties. It is involved in the regulation of lipid and carbohydrate metabolism [*Mita Y. et al., 2008*].

The function of microelements in metabolic adaptation, especially against hypoxia, is still under-investigated. The condition of microelement cerebral tissue supply under the possible hypoxia effect is also unsearched.

The aim of the paper was to study microelement cerebral tissue supply in case of experimental hypoxia with different degrees of severity.

Material and Methods

The microelement (iron, copper, zinc, manganese, chromium, and cobalt) supply, as well as the content of lead in cerebral tissues was studied under conditions of the experimental hypoxia model. Microelement supply was investigated in 44 laboratory rats on their first and seventh days; 20 intact animals made the control group. The specified time intervals correspond to neonatal and early breast-fed period of rats life span. The life conditions for rats and every experiments were carried out in line with the statements of "European Convention on Protection of Vertebrate Animals, which are Used for Experiments and Other Scientific Aims" (Strasbourg, 1986), World Medical Relief Declaration of Helsinki (2000), and "General Ethic Principles of Experiments in Animals" adopted by the First National Congress on Bioethics (Kyiv, 2001).

Experimental hypobaric hypoxia pattern was used according to methodic procedure adapted at the Division of Hypoxia States Investigation of the Institute of Physiology after A.A. Bogomolets of the National Academy of Medical Science of Ukraine. Mild anoxia was inflicted by shutting rats in a hermetic chamber for 2 hours. These rats were exposed on the 10-12th hour after birth. By means of a vacuum pump the air pressure of 525 *mm Hg* was reached; mentioned values corresponded to partial tension of 110 *mm Hg*. Carbon dioxide absorption was carried by natron lime in the hermetic chamber. Severe hypoxia was inflicted through keeping the rats of the same age (10-12th hour of life) for two hours in the hermetic chamber with 380 *mm Hg* air pressure, which corresponded to oxygen partial tension of 80 *mm Hg*. Euthanasia of animals was done by decapitation in 12 hours after withdrawal from the experiment (12 rats). Euthanasia of another 12 animals and organ specimens taking were done on the 7th day, when the rats were withdrawn from the experiment.

The obtained organs were weighted with a precision of 0.001 *g*, then the specimens were burnt in a muffle furnace at 450°C; this provided the removal of the organic matrix. When the ash was taken out, it was dissolved in a mixture of hydrochloric (2 *ml*) and nitric (1 *ml*) acids and the volume up to 10 *ml* was obtained using bidistilled water. The obtained solution was analyzed on the spectrophotometer C 15-M1 with flame atomizer ("Selmi", Ukraine). To identify the microelements a calibration diagram was plotted using standard soluble elements solutions. No less than four known concentrations were used during the calibration process. Upon the microelement identification in the solution, the sample was inserted *en*

masse and element concentration in 1*g* of studied tissue was obtained. The concentration was presented as μg*/g* of wet tissue. The measurement and calculation were done according to AAS-SPECTR program [*Tarasova I., 2013*]. The analysis and statistical processing of data were done on the Personal Computer using application programs STATISTICA 7.0 and MS Excel XP. Parametric and nonparametric statistical methods applicable for medical-and-biological research were used.

Methods of correlated-regression analysis were applied to clarify correlations between figures, the tendency in changes of their values. The two-factor analysis of variance was done to identify the fact of existence and level of monitored factors efficiency, in particular: hypoxia and animal age.

Results and Discussion

The newborn rats were characterized with a high level of iron in their cerebral tissues: 571.5±1.15 μg*/g*. Microelement level rapidly decreased in a week after their birth. It might be caused by high usage of tissue iron during oxidative reactions and energy generation processes. In this period of observation the iron level was 58.33±1.09 μg*/g*.

The correlation analysis of contained iron level in different organs of newborn animals on their first day pointed out that the level of element correlation in brain with iron levels in other organs is weak (r=-0.28 for the liver) or totally absent (r=0.12 for the kidneys and r=-0.07 for the heart). It might be possible that element content in cerebral tissues depends on blood-brain barrier permeability for albumins, which are transferring the iron. In a week, there were no correlation changes fixed in newborn rats before.

Under conditions of the experiment in mild hypoxia stage the iron content in brain tissues of newborn animals decreases almost twice, to 261.66±8.64 μg*/g*. In case of severe or complicated hypoxia the content of the microelement declines by 36.64% ($p \leq 0.05$): up to 165.67±1.23 μg*/g*. Thus, animals, which were one-week old, had the same level of element in comparison to control animal group; the level made 50.00±0.65 μg*/g* for mild hypoxia and 47.67±0.81 μg*/g* for complicated hypoxia. This phenomenon might be caused because of comprehensive blood-brain barrier functioning and sufficient adaptative possibilities for animals of this age. During hypoxia against the iron rapid loss we observed positive element content correlation between brain and liver (r=0.54) and brain and heart (r=0.49), as well as strong negative correlations between the iron level in brain and kidneys (r=-0.84).

In newborn animals mild-power converse correlations are formed between the element levels in brain and kidneys (r=-0.58) in case of severe hypoxia. Besides, the positive powerful and mild-powerful correlations between the iron content in liver, heart, and brain are still preserved. According to results of two-factor analysis of variance, the iron content at both mild and severe hypoxia changes in the wide range. The analysis for this element content in the brain demonstrated the prevailing dependence on age factor, as its efficiency was 58.33%. The degree of hypoxia effect and factors combination is almost similar and makes 21.67% and 19.45%, appropriately. These findings testify that brain iron supply is weakly related to hypoxia. Moreover, brain has high compensation abilities in early breast-fed life period of animals.

The significant copper accumulation was noticed in newborn animals. Its level in cerebral tissues reached 6.26±0.11 μg*/g*. In the end of the first week it reduced to 3.43±0.04 μg*/g*. Cerebral and kidney tissues are characterized by the least copper loss. Thus, copper content in them decreased 2-3 times, while in liver and heart tissues it decreased 5 times.

The search for correlation of copper content in newborn animals did not reveal significant interactions between its content in kidneys and heart and cerebral tissues. However, the weak negative correlation of copper level in liver and brain was noticed (r=-0.35). Possibly, copper deficit in plasma brings to its deficit in brain tissues through the element storage in liver, as this organ takes the leading function for copper metabolism. In a week, the formation of very weak negative interactions between copper content in tissues of liver and brain were observed (r=-0.21).

Mild hypoxia does not cause copper content reducing in brain tissues; however, severe hypoxia of newborn animals provokes its content decrease by 33.97% ($p \leq 0.05$). The week-old animals have copper content decline even under mild hypoxia conditions: in particular, the content of this element in

brain tissues decreases by 6.71% ($p \leq 0.05$). Upon the severe hypoxia, in the specified age group of animals further decrease in copper content is observed. Compared to mild hypoxia the analyzed element in brain decreases by 37.19% ($p \leq 0.05$).

The correlation analysis did not show interactions between the copper content in cerebral and other organ tissues under mild hypoxia of newborn animals. Under severe hypoxia, there were no changes in correlation indices.

Two-factor analysis of variance of monitored factor efficiency pointed out that copper level in brain is related to hypoxia stage (59.57%). Besides, age factor (25.7%) and mentioned factors combination (8%) have significant influence on copper concentration.

Zinc content on the first day of life was 158.54±0.66 μg/*g*, but in the end of the first week it made 109.07±0.92 μg/*g*. The weak and mild-powerful interactions were distinguished between element content in brain and liver (r=-0.23), heart (r=0.37) and kidneys (r=0.21). In a week, these interactions fainted away, and it might indicate zinc content elaboration in organs.

The mild hypoxia-related impairment causes zinc content reducing in brain of newborn rats by 16.43% ($p \leq 0.05$). In week-old animals the degree of changes in zinc content was slightly lower compared to newborns; this latter might signify to the development of adaptation mechanisms. Hence, the content of zinc in week-old animals also decreases: this time by 16.43% ($p \leq 0,05$).

The severe hypoxia extended changes in elemental composition. Mostly significant changes were observed in newborn animals. In comparison to the mild hypoxia-related affect the zinc content in cerebral tissues reduces by 43.71% ($p \leq 0.05$) on the first day, while in week-old animals it decreases by 3.89% ($p \leq 0.05$).

Under hypoxia of newborn rats the converse correlation between zinc content in brain and kidney (r=-0.62) and heart (r=-0.41) was observed. In case of severe hypoxia the positive mild-powerful correlation occurred between zinc content in cerebral and heart tissues (r=0.54).

Thus, hypoxia affect causes formation of the new correlation-based interactions between elements content in brain; this latter is due to tissue excitability changes towards the pathological factor during the neonatal period.

By means of two-factor analysis of variance it is pointed out that hypoxia stage has a minimal influence on cerebral tissue zinc content (12.1%). The age factor efficiency is 69.8%, whereas monitored factors combination has a lower effect to zinc content in brain tissues: only 17.4%. These findings explain significant changes of content in newborns and weak element reaction in week-old animals.

The content of chromium in cerebral tissues of newborn rats significantly exceeds its level in week-old animals; this fact testifies to the substantial saturation with the element. In newborn animals chromium content is 76.40±0.91 μg/*g*, in week-old animals it is 13.96±0.27 μg/*g*.

It is proved that in case of gestation the level of chromium in maternal tissues significantly decreases, its accumulation in organs of the fetus can indirectly signify to this state; the latter is confirmed by significant chromium content in brain tissues of newborn rats. Talking about chromium content, we can emphasize that mild-powerful and weak correlation of its content was identified in liver and brain (r=0.37), heart and brain (r=-0.21), kidneys and brain (r=-0.38). Thus, chromium accumulation in the internal organs has insignificant effect on its concentration in cerebral tissues of newborns.

At the end of the first week of life the loss of earlier existing correlations happened, but at the same time the new interactions were formed due to indirect changes in chromium metabolism. The main excretion pathway for the element is through the kidneys, thus enormous negative correlation between element contents in brain and kidneys is formed (r=-0.77) against the significant chromium elimination by the end of early breast-fed period. In other words, physiological element reducing in brain causes its concentration increase in kidneys, the latter is the main chromium elimination pathway from the organism.

Mild hypoxia causes chromium content decline in brain by 25.84% ($p \leq 0.05$). In case of severe hypoxia the newborns have significant chromium content reducing. The difference with the control result is 41.13% ($p \leq 0.05$). The significant minimization in chromium tissue form may identify adaptation mechanism failure in case of hard hypoxia affect.

The week-old animals have rather significant chromium content reducing even in case of mild

hypoxia affect. The difference with the control result is 26.51% ($p \leq 0.05$). The severe hypoxia in comparison to mild hypoxia affect causes chromium content reducing by 23.30% ($p \leq 0.05$). In spite of the element reducing in brain, we have not pointed out any element content correlations in brain, as well as in other studied organs. Furthermore, we have not identified any interaction between chromium content in brain and other organs in case of either mild or severe hypoxia stages.

In severe hypoxia, on the background of decreases in chromium content, there is lack of correlation in brain and other organs. The two-factor analysis of variance regarding the chromium content in animals of different age under conditions of mild and severe hypoxia revealed that the level of specified element has age factor dependence. In particular, the impact factor of the age towards the level of chromium in brain makes 76.8%. However, hypoxia degree does not influence on the level of chromium in brain, the impact factor makes 12.5%.

Unlike iron, copper and chromium, the content of cobalt is raising with the age; this latter signifies to the absence of depot for this ultramicroelement. Newborn rats have rather high level of cobalt in their cerebral tissues and it is 2.76±0.23 μg/*g*. Thus, in brain tissues the level of cobalt is rather high. For the week-old rats its level is significantly increasing to 3.26±0.31 μg/*g*, that identifies element tissue form storage. Newborn rats have also weak positive correlation between cobalt content in kidneys and brain (r=0.28). On their seventh day of live, despite the substantial content of the element in brain, we did not reveal any interaction with its level in other organs.

Due to mild hypoxia cobalt content in cerebral tissues of newborn rats reduces thrice. In case of severe hypoxia its content is decreased for another 73.54% ($p \leq 0.05$). Under mild hypoxia in a week-old animals the element content in brain is increasing by 43.55% ($p \leq 0.05$), but under the severe hypoxia affect it is reducing by 46.02% ($p \leq 0.05$).

Against the rapid element content reducing in cerebral tissues powerful positive interactions are formed between its content in brain and liver (r=0.77) and mild-powerful interactions with kidneys (r=0.46). Thus, its loss by the above-mentioned organs can cause cobalt reducing in cerebral tissues. The two-factor analysis of variance shows significant prevailing influence on cobalt content in cerebral tissues, it is 58.1%; this latter signifies to its active involvement in the microelement metabolism, though the age factor also has a reliable influence towards the cobalt content: its power makes 23.6 %, appropriately. The reliable influence on cobalt content due to combination of factors was observed only in brain tissues: 1.2%.

The manganese content is changing with time and is related to observation time. Thus, its level is reducing from 5.68±0.73 μg/*g* in newborn rats to 4.03±0.11 μg/*g* in a week-old rats. The interactions between element content were not identified: neither in brain, nor in other organs of newborns, as well as one-week old rats. Unlike other studied elements, in a week after birth the mentioned indices of correlation were preserved.

Hypoxia affect modeling causes decrease in the studied element content. Thus, mild hypoxia of newborn animals is characterized by manganese content reducing in brain tissues by 27.65% ($p \leq 0.05$). The week-old animals have also element loss in the specified tissues, reducing by 6.70% ($p \leq 0.05$) is observed.

Complicated hypoxia causes significant changes in manganese content. Newborn animals have its level reducing in comparison to mild hypoxia affect by 35.54% ($p \leq 0.05$), while the week-old rats have its reducing by 14.37% ($p \leq 0.05$). Hypoxia complication does not involve loss of established interactions.

The two-factor analysis of variance points that manganese content in cerebral tissues mostly depends on age factor, it makes 46.1%. The hypoxia efficiency level has only 29.6% of its influence. Monitored factors combination has also reliable effect on changes in its content in the brain (21.8%).

Lead content is changing as well. Thus, its content in newborn cerebral tissues is reducing from 1.17±0.21 μg/*g* to 0.44±0.09 μg/*g* in week-old animals. There are not identified correlations of lead content in other studied organs.

In case of mild hypoxia lead accumulation is observed. In cerebral tissues its content increases by 12.82% ($p \leq 0.05$). Expanding of hypoxia affect causes lead content increase against mild hypoxia affect on cerebral tissues by additional 16.66% ($p \leq 0.05$).

On the seventh day of life, due to mild hypoxia there occurs lead content raising by 15.91% ($p \leq 0.05$).

Complicated hypoxia affect causes its content increasing in cerebral tissues by 47.72% ($p \leq 0.05$).

Powerful and mild (medium-strength) correlation interactions are observed between the content of toxic element – lead – and essential elements: zinc and iron. Moreover, powerful negative interactions prevail, especially in week-old animals. Generally, the growth of correlation-related interactions power between all mentioned elements is stated in all organs of the specified age group. The explanation of these findings might be as follows: the increase of lead absolute content in organs as compared to newborn organism and its exogenous entry upon the start of enteral feeding. As known, the increase of lead content in the digestive tract brings to decreased rate of zinc and iron absorption in small intestine. Data is also available on antagonist interactions of those elements in metabolic processes.

The newborn animals have powerful and mild (medium-strength) negative correlations between the content of lead as a toxic element and the content of essential elements, zinc and iron, in case of hypoxia affect. The reducing of essential element saturation and lead content increasing cause the lead enormous competition for molecule active sites against zinc and iron. This enhances toxicity of lead.

In case of mild hypoxia, decline in positive correlation power is observed between agonist elements of brain. Therefore, interaction efficiency between copper and zinc content in brain makes only 0.12. Slightly more powerful interaction is observed between copper and manganese contents, it is from 0.39 up to 0.68. Hypoxia affect expanding involves loss of mentioned elements interactions in heart and brain. Thus, hypoxia affect causes correlation power reducing in agonist elements. The two-factor analysis of variance clarified that lead content in brain has the marked dependence on age factor (68.3%), the hypoxia affect makes 19.7%, while the influence of factors combination is not reliable.

CONCLUSION

Brain tissue of neonatal rats is characterized by high intensity and great dynamism of trace elements (iron, zinc, copper, chromium, manganese) content. By the end of the first week of life the content of these microelements decreases 1.5-10 times. The level of toxic lead is reduced more than 2.5 times. The exception to this is cobalt content, which conversely increases almost 1.2 times. Correlations of microelements content in the tissues of the brain and other organs were not found or it was too weak.

In neonatal rats mild hypoxia causes 3 times reduction of cobalt level, the level of iron decreases almost twice, manganese – by 27.65%, chromium – by 25.84%, zinc – by 16.43%. Severe hypoxia is characterized by further growth failure and imbalance of microelements, which are also more significant in early breast-fed period of animals.

Effects of mild hypoxia bring to increasing of lead accumulation in the brain by 28.2% ($p < 0.05$). In case of severe hypoxia, the content is much higher than the values obtained in the control group. The lack of linkages between the level of lead in brain, kidneys and heart was shown. Under hypoxic damage in newborn animals there remains strong negative correlation between the content of lead and zinc and iron.

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